特　許　証
(CERTIFICATE OF PATENT)

特許第４１３２６７７号
(PATENT NUMBER)

発明の名称(TITLE OF THE INVENTION)

完全型副甲状腺ホルモンの測定方法ならびに副甲状腺疾患および慢性腎不全患者の骨状態の識別方法

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この発明は、特許するものと確定し、特許原簿に登録されたことを証する。
(THIS IS TO CERTIFY THAT THE PATENT IS REGISTERED ON THE REGISTER OF THE JAPAN PATENT OFFICE.)

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特 許 庁 長 官(COMMISSIONER, JAPAN PATENT OFFICE)

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Your ref: **Our ref:**

JA42229M.GE 14 July 2008

Dear Sirs

European Patent No. EP1151307 (Application No. 00902406.8-2404)
In the Name of Scantibodies Laboratory Inc.

Reference is made to the Communication of a Notice of Opposition R. 79(1) EPC dated 4 January 2008 which set a response term of four months, subsequently extended to six months, for filing observations on the Notice of Opposition filed by Immundiagnostik AG. In response the Patentee would make the following comments.

For ease of reference the headings and paragraph numbers used by the Opponent in their Grounds of Opposition are used herein.

Reference is made herein to the opposed patent (EP1151307) and to the PCT application (WO 00/42437) from which the opposed patent is derived.

Duplicate copies of the supporting documents referred to herein as E1 to E5 accompany the postal copy of this letter. Wherein:

E1 is declaration from J. Stuart Woodhead;
E2 is Sukovaty et al., *J. Pharm. Biomed Anal.*, 42:261-271 (2006);
E3 is *Current Protocols in Immunology* (1991), § 2.1;
E4 is Fiskin et al., *J. Biol. Chem.*, 252(22):8261-8 (1977);
E5 is Kohno et al., *Journal of Clinical Laboratory Analysis*, 12:268-275 (1998).

4. Subject matter of the contested patent

It is not clear from reading the comments of the Opponent in Section 4 of the Grounds of Opposition what the actual objections to Claim 1 are, there does not appear to be any substantiated valid grounds of opposition raised. However, in view of the comments made the Patentee would make the following comments in support of Claim 1.

4.3 The present patent, EP1151307, teaches a method for producing an anti-PTH antibody, which method includes the steps of immunizing a host animal, e.g., a goat, with a complete wPTH peptide sequence, e.g., a human or rat PTH1-84 (see e.g., WO 00/42437 at page 12, lines 8-12), and affinity purification utilizing an "initial whole PTH sequence peptide" (see e.g., WO

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00/42437 at page 11, lines 8-13). One exemplary peptide that can be used in the antibody purification step is the PTH (1-8) peptide or at least 4 amino acids in the common sequence of human and rat PTH sequence (see e.g., WO 00/42437 at page 11, lines 8-13). Similarly, the PTH (1-6) peptide may be used in the affinity purification (see e.g., WO 00/42437 at pg 10 lines 4 to 14). Furthermore, Figure 2 of WO 00/42437 illustrates a wPTH assay, in which an anti-PTH antibody that binds to an epitope within (1-9) PTH sequence is used as a tracer element, i.e., a labelled antibody. This demonstrates that a PTH (1-9) fragment may be used in the affinity purification step. It is clear from the teaching of WO 00/42437 that one of a number of different peptides may be used in the affinity purification of the antibody, provided that an antibody having the claimed features is isolated. To support this, a declaration from J. Stuart Woodhead (E1) is attached. E1 demonstrates, that at the time of filing the opposed patent, the skilled person would have understood from the disclosure provided that an anti-PTH antibody with the claimed characteristics can be purified from the antisera generated from the immunization of a host animal with whole PTH1-84 using any one of a number of different initial PTH peptides. Whilst the declaration from J. Stuart Woodhead relates to US Patent Application nos: US 09/231 422 and US 09/344 639, the priority applications for the opposed patent, the comments made can be applied, by analogy, to the opposed patent. In particular, it is clear from E1 that the skilled man would have appreciated from the disclosure of WO 00/42437 that the claimed antibody could have been obtained from the antisera by affinity purification using a (1-6)PTH, (1-8)PTH or (1-9)PTH peptide.

With regard to demonstrating that an anti-PTH antibody according to the invention has the desired characteristics, WO 00/42437 states at page 13, lines 9-21:

> The present IRMA assay was compared to a conventional I-PTH immunoassay, the Allegro Nichols Intact PTH assay, (which is commercially available and made by Nichols Institute Diagnostics of San Juan Capistrano, Calif., U.S.A.), in both PTH normal patients and those suffering from chronic uremia.
>
> FIG. 5 shows the results for 34 normal human serum samples from healthy subjects which were assayed both by the present wPTH IRMA and the above I-PTH assay. In every case, the level of wPTH detected by the IRMA is lower than that reported by the I-PTH assay, demonstrating the ability of the present IRMA to avoid detecting the interfering large, non (1-84) PTH fragments detected by the I-PTH assay, (see FIGURE 11).

Figure 8 of WO 00/42437 also shows that a whole PTH assay using an exemplary antibody of the present patent can distinguish whole PTH levels in normal samples from primary hyperparathyroidism patient samples and uremic patient samples.

Therefore, contrary to the allegations in Section 4.3 of the Grounds of Opposition, WO 00/42437 teaches an actual reduction to practice of the presently claimed methods and antibodies, *i.e.*, production and purification of an exemplary antibody that was used successfully in a whole PTH

assay to detect whole PTH but not an interfering non-(1-84) parathyroid hormone fragment, *e.g.*, PTH (7-84) fragment. WO 00/42437 also demonstrates the successful use of the whole PTH assay to distinguish whole PTH levels in normal samples from primary hyperparathyroidism patient samples and uremic patient samples.

4.4 It is noted that at Section 4.4 no substantiated valid grounds of opposition are raised against any of Claims 2 to 42. In particular, lack of unity, wrong category and lack of clarity are not valid grounds of opposition.

5. Inadmissible broadening

5.2 At Section 5.2 of the Grounds of Opposition the Opponent asserts that Claim 1 of the opposed patent has been broadened to extend beyond the application as filed. In response the Patentee would comment that all elements of the presently claimed invention were disclosed in the application as filed – see WO 00/42437.

WO 00/42437 teaches all elements of the presently claimed methods and antibodies, to demonstrate this each section of Claim 1 is considered in turn

> "A method for measuring the amount of whole parathyroid hormone in a sample, while not detecting an interfering non-(1-84) parathyroid hormone fragment"

Basis for the preamble of Claim 1 which recites a "method for measuring the amount of whole parathyroid hormone in a sample" can be found at page 5, lines 1-5 of WO 00/42437:

> One detects the level in the serum or blood of at least one of three different parameters, namely, whole or non-fragmented parathyroid hormone in a biological sample, a large non-whole parathyroid hormone peptide fragment that can function as a parathyroid hormone antagonist, or the combination of the two values. (emphases added.)

Basis for this recitation can also be found in the original Claims 7, 31, and 44 of WO 00/42437, all of which are directed to methods for "determining one parameter selected from the group consisting of the whole parathyroid hormone level, the parathyroid hormone inhibitory peptide fragment level, and a calculated total parathyroid hormone level." (emphases added.)

Basis for the preamble reciting "not detecting an interfering non-(1-84) parathyroid hormone fragment" can be found at various places in WO 00/42437. For example, WO 00/42437, at page 2, lines 5-19, teaches:

> The complete form of human PTH, (hPTH), is a unique 84 amino acid peptide (SEQ ID NO. 2), as is shown in FIG. 1. Researchers have found that this peptide has an anabolic effect on bone that involves a domain for protein kinase C activation (amino acid residues 28 to 34) as well as a domain for adenylate cyclase

> activation (amino acid residues 1 to 7). However, various catabolic forms of clipped or fragmented PTH peptides also are found in circulation, most likely formed by intraglandular or peripheral metabolism. For example, whole PTH can be cleaved between amino acids 34 and 35 to produce a (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment. Likewise, clipping can occur between either amino acids 36 and 37 or 37 and 38. Recently, a large PTH fragment referred to as "non-(1-84) PTH" has been disclosed which is clipped closer to the N-terminal end of PTH. (See R. LePage el alia, "A non-(1-84) circulating parathyroid hormone (PTH) fragment interferes significantly with intact PTH commercial assay measurements in uremic samples " Clin Chem (1998); 44: 805-810.)

Therefore, WO 00/42437 makes it clear that the "non-(1-84) PTH" fragment is generated by cleavage at a site that is closer to the N-terminal end of PTH relative to the cleavage site between positions 34 and 35, which generates the previously known (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment.

WO 00/42437 also teaches that "[t]he present invention incorporates a discovery that a large, non-whole PTH peptide fragment, a peptide having an amino acid sequence from between (SEQ ID No. 2 [PTH3-84]) and (SEQ ID No. 3 [PTH34-84]), functions in vivo as a wPTH antagonist or inhibitor (PIN), (see FIG. 12)" (WO 00/42437 at page 5, lines 11-14). WO 00/42437 further teaches that "[i]n making a measurement of wPTH, one does not want to detect PIN." (WO 00/42437 at page 5, line 24.)

> "a first antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment"

Basis for the "first antibody" can be found at page 5, line 24 through page 6, line 1 of WO 00/42437:

> The method for measuring the amount of wPTH in a sample such as serum, plasma, or blood comprises four general steps which can vary depending upon whether one uses a first antibody or antibody fragment specific for the PTH peptide SER-VAL-SER-GLU-ILEGLN-LEU-MET (SEQ ID No. 4), wherein at least four amino acids are part of the antibody reactive portion of the peptide either as a signal antibody or a capture antibody in conventional immunoassay formats.

"which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen"

Basis for this recitation can be found at page 12, lines 8-12 of WO 00/42437:

> To create an affinity-purified anti-(1-6) PTH antibody, one first uses a selected initial PTH sequence peptide as described above as part of an immunogen for injection into a goat. The peptide can be used either by itself as an injectible immunogen, incorporated into a non PTH peptide having a molecular weight, typically, of between about 5000 and 10,000,000, or as part of the wPTH complete sequence. (emphases added.)

"adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the initial parathyroid hormone peptide sequence which binds to the first antibody"

Basis for this recitation can be found at page 6, lines 5-10 of WO 00/42437:

> A specific binding label comprised of a second antibody and a conventional immunoassay label, such as chemiluminescent agents, colorimetric agents, energy transfer agents, enzymes, fluorescent agents, and radioisotopes, is used to label the complex, preferably at the C-terminal end of wPTH, and can be added either substantially simultaneously with the first antibody or subsequent thereto.

"either the first antibody or antibody fragment or the second antibody or antibody fragment is labelled"

WO 00/42437 teaches that either the first or the second antibody can be used as a labelled or signal antibody. (*See* WO 00/42437 at page 5, line 24 through page 6, line 1, and page 6, lines 11-13.)

"measuring the amount of the labelled complex to measure the amount of whole parathyroid hormone in the sample"

Basis for this recitation can be found at page 6, lines 5-10 of WO 00/42437:

> Finally, one uses conventional techniques to measure the amount of labeled complex, and thereby calculate wPTH levels in the sample.

The Opponent's allegation with regard to Claim 34 is without merit because the limitation in Claim 34 finds basis in WO 00/42437 at page 5, lines 11-14.

5.3 The Opponent's allegation that the "interfering non-(1-84) parathyroid hormone fragment" is not a defined concept is wrong. WO 00/42437 makes it clear that the "non-(1-84) PTH" fragment is generated by cleavage at a site that is closer to the N-terminal end of PTH relative to the cleavage site between positions 34 and 35, which generates the previously known (1-34) PTH N-terminal fragment and a (35-84) PTH C-terminal fragment (WO 00/42437 at page 2, lines 5-19). WO 00/42437 also teaches that "[t]he present invention incorporates a discovery that a large, non-whole PTH peptide fragment, a peptide having an amino acid sequence from between (SEQ ID No. 2 [PTH3-84]) and (SEQ ID No. 3 [PTH34-84]), functions in vivo as a wPTH antagonist or inhibitor (PIN), (see FIG. 12)" (WO 00/42437 at page 5, lines 11-14).

5.4 The Opponent's allegation that the invention originally applied for was the measurement of at least two parameters and only this method is supported by the description of WO 00/42437 is incorrect. Original Claims 7, 31, and 44 of WO 00/42437 are all directed to methods for "determining one parameter selected from the group consisting of the whole parathyroid hormone level, the parathyroid hormone inhibitory peptide fragment level, and a calculated total parathyroid hormone level" (emphases added) (WO 00/42437 at page 21, lines 23-27; page 24, lines 18-21; and page 26, lines 11-14).

The Opponent's other allegation that "the exclusion of all interfering factors and fragments through the use of special antibodies was not disclosed" is also wrong. WO 00/42437 teaches that the "non-(1-84) PTH" fragment to be avoided in the whole PTH assay is generated by a cleavage at a site that is closer to the N-terminal end of PTH relative to the cleavage site between the positions 34 and 35 (WO 00/42437 at page 2, lines 5-19). WO 00/42437 also teaches that "[t]he present invention incorporates a discovery that a large, non-whole PTH peptide fragment, a peptide having an amino acid sequence from between (SEQ ID No. 2 [PTH3-84]) and (SEQ ID No. 3 [PTH34-84]), functions in vivo as a wPTH antagonist or inhibitor (PIN), (see FIG. 12)." (WO 00/42437 at page 5, lines 11-14.) The WO 00/42437 further teaches that "[i]n making a measurement of wPTH, one does not want to detect PIN" (WO 00/42437 at page 5, line 24).

5.5 The Opponent's comment that the originally submitted application does not contain any concrete indications regarding how the claimed first antibodies could be obtained, and, in particular, it is asserted that the only disclosure in the application as originally filed is that a synthetic 1-8 peptide must be used as the immunogen. In response, the Patentee would draw the Opposition Division's attention to the Patentee's comments made with reference to Sections 4.3 and 5.2.

5.6 The Opponent's allegation that the compulsory use of the whole wPTH sequence as the immunogen, as presented in Claim 1, is not disclosed in the original application is without merit. In particular, please refer to the discussion at Section 5.2 above.

The Opponent's allegation that the affinity-purified "(1-6)-PTH antibodies" described bind antagonistic PTH (3-84) in every case is a conclusory statement without any supporting evidence. In fact, experimental evidence shows that an exemplary antibody of the presently claimed

invention does not detect a PTH (3-84) fragment. As shown by Sukovaty et al., *J. Pharm. Biomed Anal.*, 42:261-271, 266-267 (2006) (E2), Scantibodies' commercial PTH assay (kit B), in which the labelled antibody is produced by immunization with the whole wPTH and affinity purified with a PTH (1-9) peptide, is shown to be specific for whole PTH and to avoid binding to the PTH (3-84) or PTH (7-84) fragment. For example, E2 states at page 267, left col:

> Kit B [Scantibodies' commercial PTH assay] results indicated no observable interference by PTH (3-84) or PTH (7-84) at all concentrations tested. These results supported the cross-reactivity results and confirmed the Kit B was the most specific for PTH (1-84) quantitation.

The rest of the allegations in Section 5.6 are also wrong and are based on an incorrect interpretation of the claim language. The present claims, *e.g.*, Claims 1 and 37, recite an "antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment." The claimed antibody must therefore bind to at least four amino acids in PTH (1-8) sequence, but contrary to the Opponent's allegation, the claimed antibody is not limited to binding to only four amino acids in the PTH (1-8) sequence. The claimed antibody can also bind to five, six, seven or eight amino acids in PTH (1-8) sequence. The claimed antibody is specific for the PTH (1-8) sequence as part of the whole parathyroid hormone (wPTH) complete sequence because the antibody is produced by immunization with a wPTH complete sequence and binds to at least four amino acids in the PTH (1-8) sequence.

5.7 It is noted that at Section 5.7 no substantiated valid grounds of opposition are raised against any of Claims 2 to 42.

The Opponent raises a specific allegation with regard to Claim 34. However, basis for this claim can be found in WO 00/42437 which teaches that "[t]he present invention incorporates a discovery that a large, non-whole PTH peptide fragment, a peptide having an amino acid sequence from between (SEQ ID No. 2 [PTH3-84]) and (SEQ ID No. 3 [PTH34-84]), functions in vivo as a wPTH antagonist or inhibitor (PIN), (see FIG. 12)" (WO 00/42437 at page 5, lines 11-14). WO 00/42437 further teaches that "[i]n making a measurement of wPTH, one does not want to detect PIN" (WO 00/42437 at page 5, line 24).

The Opponent also raised a specific allegation with regard to Claim 13. However, basis for this claim can be found in the testing data shown in Table 2, page 19 of WO 00/42437.

6 Insufficient disclosure

6.1 Contrary to the Opponent's allegation, the present patent teaches various embodiments for obtaining the presently claimed antibody. For example, WO 00/42437 teaches at page 11, lines 7-13; emphases added:

> In order to make the signal antibody in the above assay, first one makes a synthetic PTH peptide corresponding either to hPTH(Ser-Val-Ser-Glu-Ile-Gln-Leu-Met), rat PTH(Ala-Val-Ser-Glu-Ile-Gln-Leu-Met), or at least four amino acids in the common sequence. The selected peptide can play two roles in making an assay, first as a specific source for creating a polyclonal antibody or monoclonal antibody source for signal antibody or capture antibody, and second as part of an affinity purification means for isolating the desired signal antibody or capture antibody.

Furthermore the skilled man would be readily able to ascertain whether any antibody obtained had the claimed properties. For example, if an antibody is affinity purified with a peptide of four amino acids the purified antibody binds to an epitope contained within the four amino acids. This means that the epitope can contain all four amino acids or less than the four amino acids. If there is a need to determine whether an antibody purified by a peptide of four amino acids will bind to an epitope made entirely of three amino acids, one skilled in the art can perform an immunoassay to test if the antibody will bind to a peptide of three amino acids using a commonly known immunoassay format, *e.g.*, indirect ELISA assay and double antibody-sandwich assay, as taught in *Current Protocols in Immunology* (1991), § 2.1, especially §§ 2.1.1-2.1.6 and §§ 2.1.11-2.1.13 (E3). There would be no undue burden on one skilled in the art to determine whether an antibody would bind to a peptide of three amino acids.

The other allegation that the first antibodies would undoubtedly bind interfering antagonistic PTH fragments such as PTH 3-84 (SEQ ID No. 2) and thus is somehow contradictory with the negative method feature "while not detecting an interfering non-(1-84) parathyroid hormone fragment" is a conclusory statement without any supporting evidence. As discussed at Section 5.6, the fact is that an exemplary antibody of the presently claimed invention does not detect a PTH (3-84) or PTH (7-84) fragment.

6.2 As discussed in detail in connection with Section 4.3, contrary to the allegations in Section 6.2, WO 00/42437 teaches an actual reduction to practice of the presently claimed methods and antibodies, i.e., teaches the production and purification of an exemplary antibody that was used successfully in a whole PTH assay to detect whole PTH but not an interfering non-(1-84) parathyroid hormone fragment, e.g., PTH (7-84) fragment. Furthermore, this successful whole PTH assay can distinguish whole PTH levels in normal samples from primary hyperparathyroidism patient samples and uremic patient samples.

6.3 The allegation that the claimed invention was not contained in the original application, or not described in the contested patent in such a manner that a person skilled in the art could realise it is without merit. See the above discussion in connection with Sections 4.3 and 5.2. The alleged detection of PTH (3-84) fragment by the presently claimed antibody is simply wrong. See the above discussion in connection with Section 5.6. The further allegation on the requirement that the presently claimed antibody bind to at least four amino acids in the PTH (1-8) sequence is also wrong for the reasons discussed with reference to Section 6.1.

6.4 The allegation that there is no actual relationship between the claimed method according to Claim 1 and the clinical tests is without merit. As indicated in paragraph [0031] of the present patent (also WO 00/42437 at page 13, lines 16-24.) :

> FIGURE 5 shows the results for 34 normal human serum samples from healthy subjects which were assayed both by the present wPTH IRMA and the above I-PTH assay. In every case, the level of wPTH detected by the IRMA is lower than that reported by the I-PTH assay, demonstrating the ability of the present IRMA to avoid detecting the interfering large, non (1-84) PTH fragment detected by the I-PTH assay, (see FIGURE 11). FIGURE 6 illustrates how such interference can occur. An N-terminal PTH specific signal antibody which is not specific to the initial PTH peptide sequence, as in the present invention, can detect not only wPTH (as in the upper part of FIGURE 6), but also can detect PIN, the large, non (1-84) PTH fragment, (as in the lower part of FIGURE 6).

As made clear here, the critical difference between the wPTH assay of the present patent and the previously known I-PTH assay is that the present wPTH assay avoids detecting the interfering large, non (1-84) PTH fragment detected by the I-PTH assay.

Paragraph [0031] further points to Figures 6 and 11 to illustrate the important characteristics of the present wPTH assay. As shown in Figure 6, the reason that the previously known I-PTH assay cannot accurately measure the whole PTH level in samples from normal people or patients is that the antibody used in the I-PTH assay binds to an epitope that exists both in the whole PTH and in an interfering large, non (1-84) PTH fragment, *e.g.*, PTH (7-84) fragment. Accordingly, WO 00/42437 teaches that the "method for measuring the amount of wPTH in a sample such as serum, plasma, or blood comprises four general steps which can vary depending upon whether one uses a first antibody or antibody fragment specific for the PTH peptide SER-VAL-SER-GLU-ILEGLN-LEU-MET (SEQ ID No. 4), wherein at least four amino acids are part of the antibody reactive portion of the peptide either as a signal antibody or a capture antibody in conventional immunoassay formats" (WO 00/42437 at page 5, line 24 through page 6, line 1).

In addition, as shown in Figures 2 and 3, to be used in the presently claimed methods for measuring the amount of whole parathyroid hormone in a sample, the antibody must be specific

for PTH (1-8) sequence, not alone, but as part of the whole wPTH complete sequence, as required by the claims of the present patent.

Further, the whole parathyroid hormone in samples from a clinical source, *e.g.*, blood samples from normal people or patients, may exist in its natural form, including any natural three dimensional structures. Fiskin et al., *J. Biol. Chem.*, 252(22):8261-8 (1977) (E4) analyzed images of parathormone obtained by dark field electron microscopy in order to determine the three-dimensional structure of the molecule. Based on their analysis, E4 et al. postulated a model for the PTH three dimensional structure or conformation. (*See* Figure 6 of E4 at page 8267, and page 8265, right col.) As shown in the model depicted in Figure 6 of E4, the PTH (1-8) or PTH (1-9) amino acid residues form an alpha-helix three dimensional structure or conformation. Therefore, in order to measure the amount of whole parathyroid hormone in the clinical samples, as recited in Claim 14 of the present patent, one skilled in the art would understand that it is preferable to use an antibody that recognizes the three dimensional structure or conformation in the PTH (1-6), PTH (1-8) or PTH (1-9) as existed in the whole PTH. This is taught in the present application (*See e.g.*, WO 00/42437 at page 12, lines 8-12) and recited in the claims of the present patent.

7 Novelty

7.1 D1 (Tampe) The methods and antibodies claimed in the opposed patent require an "antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence" and that the "antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen." D1 does not disclose a method or antibody with these features. In particular, the features relating to the whole PTH are not specifically disclosed in D1, and the skilled man upon reading D1 would appreciate that the methods and antibodies described in D1 would not result in methods or antibodies meeting the requirements of the methods and antibodies claimed in the opposed patent.

D1 discloses the production of both polyclonal and monoclonal antibodies to fragments of hPTH. Regarding the production of the polyclonal antibodies, D1 states at page 3:

> A polyclonal antiserum against the N-Terminal part of PTH was raised by immunizing a goat with extract from human adenomatous parathyroid glands. After repeated booster immunization, a specific antiserum for hPTH(1-34) was obtained.

Furthermore, the experimental evidence disclosed in D1 indicates that the actual immunogen used in D1 is the (1-34) PTH N-terminal fragment, not the whole PTH. The polyclonal antiserum raised with the human adenomatous parathyroid glands, without any affinity purification, binds to the (1-34) PTH N-terminal fragment, but does not bind to hPTH (44-68) and hPTH (53-84) fragments (D1 at page 5). This indicates that the "real" immunogen used in D1 for producing the polyclonal antiserum was the (1-34) PTH N-terminal fragment. If the whole PTH were the "real" immunogen, the polyclonal antiserum would bind to the hPTH (44-68) and hPTH (53-84)

fragments. Perhaps recognizing that this was the case, *i.e.*, the "real" immunogen used for producing the polyclonal antiserum was the (1-34) PTH N-terminal fragment, D1 states that a "polyclonal antiserum against the N-Terminal part of PTH was raised" and "a specific antiserum for hPTH(1-34) was obtained" (D1 at page 3).

The monoclonal antibodies in D1 were produced using a PTH (1-38) fragment as the immunogen (D1 at page 3) and not the whole PTH as required by the methods and antibodies of the opposed patent. As discussed above in connection with the polyclonal antiserum, such immunization in D1 does not produce an antibody that binds to the whole PTH, let alone an antibody that must be specific for the PTH (1-8) sequence as part of whole parathyroid hormone (wPTH) complete sequence. Therefore the monoclonal antibodies raised by immunization with PTH (1-38) fragment in D1 do not anticipate the presently claimed methods and antibodies.

To further support the assertion that the methods of D1 did not produce antibodies which fall within the scope of the claims of the opposed patent, the patentee would draw attention to the teaching of Kohno et al., in the *Journal of Clinical Laboratory Analysis*, 12:268-275 (1998) (E5). E5 teaches the production of an antibody generated by immunization and purification with hPTH (1-34) (E5 at pages 269-27). Through epitope mapping of the antibodies produced E5 identifies a PTH (4-10) antibody (E5 at page 271). E5's antibody, however, is not specific for whole PTH and acknowledges that its "assay is specific for PTH (1-34) and practically no interference occurred with PTH (1-84) up to 300 pg/ml." (E5 at page 272, left column and page 274). Therefore, by analogy to the teaching of E5, the antibodies produced in D1 did not recognise whole PTH and therefore do not anticipate the presently claimed methods and antibodies.

Further, D1 does not disclose the additional limitations of many of the dependent claims, *e.g.*, Claims 12-36 and Claims 40-42. Accordingly, D1 does not anticipate these dependent claims.

Novelty and Inventive Step over D2, D3, D4, D8 and D10

The Opponent's allege that D10 (WO 1996/010041A1, EP 0 783 522 B1, US 6,030,790), D2 (Mägerlein M et al, rzneim.-Forsch./Drug Res., 1998, 48 (I), 199-204) and D3 (Mägerlein M et al., Arzneim.-Forsch./Drug Res., 1998, 48(II), 738-787) anticipate the presently claimed methods and antibodies, because these references describe antibodies against an hPTH(1-8) sequence.

D2, D3 and D10 do not anticipate the presently claimed methods and antibodies for numerous reasons. The presently claimed methods and antibodies recite "antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence" and the "antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen."

In stark contrast, D2, D3 and D10 disclose or teach the use of short N-terminal PTH peptides for immunizing a mammal to generate anti-PTH antibodies. In fact, these references teach away from immunizing with whole PTH. For example, U.S. patent No. 6,030,790 (D10), Mägerlein being one of the listed inventors, states at col. 2:4-31:

> When immunizing using the intact antigen, a heterogeneous mixture of various antibodies is obtained, which first must be subjected to an expensive purification in order to conduct a sandwich assay. . . .
>
> The technical problem which this invention is based upon is to provide peptides by means of which it is possible to eliminate the above-mentioned drawbacks in the diagnosis of biologically active hPTH.
>
> Surprisingly, the technical problem described above is solved by means of the following peptides from the sequence of hPTH(1-37): [citation of various PTH peptides omitted].

According to D10, using an intact antigen, *e.g.*, the whole PTH, as an immunogen is problematic and D10's solution to solve the problem is to use short PTH peptides as the immunogen. Therefore, D2, D3 and D10 do not teach or even suggest the use of the whole PTH as the immunogen to generate anti-PTH antibodies, indeed, they actually teach away from such use.

As discussed above in connection with D1, immunization with the (1-34) PTH N-terminal fragment does not necessarily produce an antibody that binds to the whole PTH, let alone an antibody that must be specific for the PTH (1-8) sequence as part of whole parathyroid hormone (wPTH) complete sequence. Given that the immunogen used in D2, D3 and D10, *e.g.*, hPTH (1-10) peptide, is much smaller than the one used in E5, hPTH (1-34) peptide, it is more likely that the antibody in D2, D3 and D10 would not bind to the whole PTH. Therefore, in view of the common knowledge in the art, *e.g.*, teachings in E5, the antibodies raised by immunization with small PTH peptides, *e.g.*, hPTH (1-10) peptide, in D2, D3 and D10 do not anticipate the presently claimed methods and antibodies.

The Opponents also allege that D4 (Logue et al) and D8 (Fisher et al.) disclose antibodies, which bind epitopes within the region of hPTH(1-8) or hPTH(1-6) and sandwich immunoassays with these antibodies without any supporting evidence and/or analysis.

D4 discloses immunization with PTH (1-10) and PTH (1-34) peptides (D4 at page 160). The immunization with PTH (1-10) failed to produce any antibody (D4 at page 161). Certain immunization with PTH (1-34) produced antibodies. The presently claimed methods and antibodies recite the "antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen." D4 fails to disclose this limitation.

In addition, D4 did not affinity purify and/or characterize the binding specificity of the produced antibodies. Without such affinity purification and/or characterization, D4 does not show the features recited in the presently claimed methods and antibodies that the "antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment," and "not detecting an interfering non-(1-84) parathyroid

hormone fragment." Therefore, D4 does not anticipate the presently claimed methods and antibodies.

D8 discloses immunizing goats with a urea-trichloroacetic acid extract of human parathyroid tumours [(hPTH-(TCA)], synthetic hPTH (1-12) peptide, synthetic hPTH (1-34) peptide and purified bovine PTH (1-84) [bPTH (1-84)] (D8, the "*Peptides*" Section at pages 1383-1384, and the "*Immunization and quantification of antibodies*" Section at page 1385). After the sera were collected, the "sera were heat inactivated (56°C, 30 min) and stored in small portions at - 20°C until use." (*Id.*) The sera or antisera were tested without any isolation or purification. (*Id.*)

The presently claimed methods and antibodies recite the "antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen." The antisera raised with synthetic hPTH (1-12) peptide and synthetic hPTH (1-34) peptide in D8 fail to disclose this limitation.

In addition, the hPTH (1-84), *i.e.*, the hPTH-(TCA), and bPTH (1-84) used in D8 to generate antisera were crude extracts and/or not sufficiently purified. "The estimated purity [of the hPTH-(TCA)] was 10%" and the bPTH (1-84) "was assumed to have a purity of 79%." (D8, the "*Peptides*" Section at pages 1383-1384). It is well recognized in the art that parathyroid tissue or cells produce both whole PTH and other non-(1-84) parathyroid hormone (PTH) fragments or amino-terminal (N) truncated fragments. (*See e.g.*, WO 00/42437 at page 2, lines 12-19.) Because the crude or not sufficiently purified parathyroid tissue or cell extract was used in D8, it is expected that the antisera generated using the extract would contain antibodies that would bind to the non-(1-84) PTH fragments. Accordingly, the antisera generated by immunization with the hPTH-(TCA) and the bPTH (1-84) would not meet the limitations that the "antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment" and "not detecting an interfering non-(1-84) parathyroid hormone fragment." Therefore, D8 does not anticipate the presently claimed methods and antibodies.

Furthermore, D4 or D8 do not disclose the additional limitations of many of the dependent claims, *e.g.*, Claims 12-36 and Claims 40-42. Accordingly, neither D4 nor D8 anticipate these dependent claims.

8. Inventive step

The Opponent's allegation that the presently claimed methods and antibodies lack an inventive step in view of D11 (Le Page) is erroneous because D11, whether alone or in combination with other prior art references, does not teach or suggest the presently claimed methods and antibodies. The fundamental difference between the present patent and D11 is that D11 uses HPLC, not an immunoassay, to distinguish whole parathyroid hormone from the non-(1-84) parathyroid hormone fragment. Therefore, D11 does not teach any of the recited limitations of the presently claimed methods and antibodies, for example, an "antibody or antibody fragment that is specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID

NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment" and "not detecting an interfering non-(1-84) parathyroid hormone fragment."

D11 simply reinforces the problem solved by the invention claimed in the opposed patent, namely that an assay is needed for the detection of whole PTH (1-84) which does not also detect an interfering non-(1-84) parathyroid hormone fragment. The invention solves this problem by using an antibody specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, wherein the whole PTH molecule was used as the immunogen. This solution is neither taught nor suggested by any of the prior art documents, either alone or in combination.

In particular, none of the other cited prior art references, D5, D7 and D10, cures the deficiencies in the PTH assays studied in D11, and furthermore none of these references teach the requisite limitations of the presently claimed methods and antibodies.

In addition, the only cited reference that teaches antibodies that are supposed to bind to the very N-terminus of PTH, D10, teaches away from the presently claimed methods and antibodies. As discussed above, according to D10, using an intact antigen, *e.g.*, the whole PTH, as an immunogen is problematic and D10's solution to solve the problem is to use short PTH peptides as immunogen. Therefore, D11, D5 and D7, in view of D10, do not teach or even suggest the use of the whole PTH as an immunogen to generate anti-PTH antibodies, and actually teach away from such use. Therefore, the presently claimed methods and antibodies involve an inventive step over D11 and the other cited prior art references.

Further, D11, whether alone or in combination with other prior art references, does not teach or suggest the additional limitations of many of the dependent claims, *e.g.*, Claims 12-36 and Claims 40-42. Therefore, the dependent claims involve an inventive step over D11 and the other cited prior art references.

9. Request for the reimbursement of the opposition fee

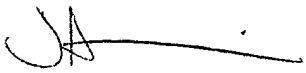
The Patentee can see no basis in the European Patent Convention for the opposition fee to be refunded to the Opponent.

Conclusion

In view of the foregoing comments the Patentee respectfully submits that the claims granted on the opposed patent are allowable, and we therefore make our main request the maintenance of the opposed European patent as granted. However, should the Opposition Division be not prepared to maintain the claims, the enclosed five auxiliary claims are submitted. It is intended that combinations of the amendments made in the five auxiliary requests may also be considered.

In the event that the Opposition Division does not intend to allow the patent to be maintained as granted, oral proceedings are hereby requested.

Yours faithfully



Jennifer Atkinson – Professional Representative
for and on behalf of Barker Brettell LLP

enc

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Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
            20                  25                  30
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Claims

1. A method for measuring the amount of whole parathyroid hormone in a sample while not detecting an interfering non-(1-84) parathyroid hormone fragment, said method **characterized by**:

   a) adding to the sample a first antibody or antibody fragment specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment; which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen;

   b) adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the initial parathyroid hormone peptide sequence which binds to the first antibody, wherein either the first antibody or antibody fragment or the second antibody or antibody fragment is labeled, thereby forming a labeled complex; and

c) measuring the amount of the labeled complex to measure the amount of whole parathyroid hormone in the sample.

2. The method of claim 1 wherein the second antibody or antibody fragment is added sequentially or simultaneously with the first antibody or antibody fragment.

3. The method of claim 1 wherein the first antibody or antibody fragment is bound to a solid support.

4. The method of claim 3 wherein the first antibody or antibody fragment is bound to a colloidal solid support.

5. The method of claim 4 wherein the colloidal solid support is latex particles.

6. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a monoclonal antibody.

7. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a polyclonal antibody.

8. The method of claim 1, wherein the second antibody or antibody fragment is labeled.

9. The method of claim 1 wherein the second antibody or antibody fragment is bound to a solid support.

10. The method of claim 1 wherein the label of the labeled antibody or antibody fragment is selected from the group consisting of a chemiluminescent agent, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

11. The method of claim 1, wherein the first antibody or antibody fragment is a goat anti-(1-6) parathyroid hormone antibody.

12. The method of claim 1, wherein the method is capable of detecting wPTH at a normal physiological level.

13. The method of claim 1, wherein the method is capable of detecting wPTH at levels of 27.89 pg/ml and below.

14. The method of claim 1, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

15. The method of claim 1, further comprising the step of determining either the level of total PTH or the level of parathyroid hormone inhibitory peptide fragment or the level of both in the sample.

16. The method of claim 15, wherein the level of parathyroid hormone inhibitory peptide fragment in the sample is determined by subtracting the measured level of whole PTH in the sample from the measured level of total PTH in the sample to calculate the level of parathyroid hormone inhibitory peptide fragment.

17. The method of claim 15 or 16, wherein total PTH level is determined using an antibody specific for the fragment PTH_{7-38}.

18. The method of claim 15 or 16, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby determining whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

19. The method of claim 18, wherein the parathyroid disease is primary hyperparathyroidism.

20. The method of claim 18, wherein the parathyroid disease is secondary hyperparathyroidism.

21. The method of claim 18, wherein the parathyroid disease is caused by chronic renal failure.

22. The method of claim 18, wherein the parathyroid disease is renal osteodystrophy.

23. The method of claim 22, wherein the renal osteodystrophy is selected from the group consisting of osteitis fibrosa

cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

24. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the parathyroid hormone inhibitory peptide fragment level.

25. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the total parathyroid hormone level in the sample.

26. The method of Claim 18 wherein the parathyroid hormone inhibitory peptide fragment level is compared with the total parathyroid hormone level in the sample.

27. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level,
thereby monitoring parathyroid related bone disease and treatment in the person from whom the sample was collected.

28. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby monitoring effects of the therapeutic treatment for hyperparathyroidism in the person from whom the sample was collected.

29. The method of claim 28, wherein the hyperparathyroidism is selected from the group consisting of primary hyperparathyroidism, secondary hyperparathyroidism, renal bone disease, renal osteodystrophy, osteitis fibrosa cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

30. The method of any of claims 15-17, further comprising the step of comparing the whole parathyroid hormone level with the parathyroid hormone inhibitory peptide fragment level to monitor renal osteodystrophy and its treatment.

31. The method of any of claims 18-30 wherein the comparison is in the form of a ratio or proportion.

32. The method of claim 18, wherein the sample is from a person who is a patient with chronic uremia.

33. The method of claim 31, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

34. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence from between PTH_{3-84} (SEQ ID NO:2) and PTH_{34-84} (SEQ ID NO:3) and functions *in vivo* as a parathyroid hormone antagonist or inhibitor (PIN).

35. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

36. The method of claim 1, further comprising the step of using the level of whole parathyroid hormone in the sample to determine whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

37. A substantially pure antibody or antibody fragment specific for an initial peptide sequence of whole parathyroid hormone, wherein the initial peptide sequence consists of SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO: 4) as part of wPTH, wherein at least four amino acids in this sequence are part of a reactive portion with the antibody or antibody fragment, and wherein the antibody or antibody fragment is produced using the complete wPTH peptidic sequence as an immunogen, and wherein the antibody or antibody fragment does not detect an interfering non-(1-84) parathyroid hormone fragment.

38. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a monoclonal antibody.

39. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a polyclonal antibody.

40. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of

detecting wPTH at a normal physiological level.

41. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of detecting wPTH at levels of 27.89 pg/ml and below.

42. The antibody or antibody fragment of any of claims 37-41, or the method of any of claims 1 to 36, wherein the first antibody or antibody fragment is affinity purified using a synthetic peptide selected from hPTH1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET), rat PTH 1-8 (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)), or a peptide of at least four amino acids in the common sequence.

Patentansprüche

1. Verfahren zum Messen der Menge an vollständigem Parathormon in einer Probe, während ein interferierendes Nicht-(1-84)-Parathormonfragment nicht detektiert wird, wobei das Verfahren **gekennzeichnet ist durch:**

   a) Zugabe zur Probe eines ersten Antikörpers oder Antikörperfragments, welches für das Parathormonpeptid SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil der Komplettsequenz des vollständigen Parathormons (wPTH) spezifisch ist, und wobei zumindest vier Aminosäuren in dem Peptid Teil eines reaktiven Bereichs des ersten Antikörpers oder Antikörperfragments sind, wobei der erste Antikörper oder das erste Antikörperfragment unter Verwendung der kompletten wPTH-Peptidsequenz als ein Immunogen hergestellt wird,
   b) Zugabe eines zweiten Antikörpers oder Antikörperfragments, der/das spezifisch an einem anderen Teil des vollständigen Parathormons als die anfängliche Parathormon-Peptidsequenz, die an den ersten Antikörper bindet, bindet,
   wobei entweder erster/s Antikörper oder Antikörperfragment oder der zweite/s Antikörper oder Antikörperfragment markiert ist,
   wodurch ein markierter Komplex gebildet wird; und
   c) Messen der Menge des markierten Komplexes, um die Menge an vollständigem Parathormon in der Probe zu messen.

2. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment sequentiell oder gleichzeitig mit dem ersten Antikörper oder Antikörperfragment zugegeben wird.

3. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment an einem festen Träger gebunden ist.

4. Verfahren nach Anspruch 3, wobei der/das erste Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

5. Verfahren nach Anspruch 4, wobei der kolloidale, feste Träger Latexpartikel ist.

6. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein monoklonaler Antikörper ist.

7. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein polyklonaler Antikörper ist.

8. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment markiert ist.

9. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

10. Verfahren nach Anspruch 1, wobei die Markierung des markierten Antikörpers oder Antikörperfragments ausgewählt ist aus der Gruppe, die aus einem Chemilumineszenzagens, einen kolorimetrischen Agens, einem Energieübertragungsagens, einem Enzym, einem Fluoreszenzagens und einem Radioisotop besteht.

11. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment ein Ziegen-anti-(1-6)-Para-

SECOND AUXILIARY REQUEST


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Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
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Claims

1. A method for measuring the amount of whole parathyroid hormone in a sample while not detecting an interfering non-(1-84) parathyroid hormone fragment, said method **characterized by:**

   a) adding to the sample a first antibody or antibody fragment specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment; which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen; Synthetic

   b) adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the initial parathyroid hormone peptide sequence which binds to the first antibody, wherein either the first antibody or antibody fragment or the second antibody or antibody fragment is labeled, thereby forming a labeled complex; and

c) measuring the amount of the labeled complex to measure the amount of whole parathyroid hormone in the sample.

2. The method of claim 1 wherein the second antibody or antibody fragment is added sequentially or simultaneously with the first antibody or antibody fragment.

3. The method of claim 1 wherein the first antibody or antibody fragment is bound to a solid support.

4. The method of claim 3 wherein the first antibody or antibody fragment is bound to a colloidal solid support.

5. The method of claim 4 wherein the colloidal solid support is latex particles.

6. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a monoclonal antibody.

7. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a polyclonal antibody.

8. The method of claim 1, wherein the second antibody or antibody fragment is labeled.

9. The method of claim 1 wherein the second antibody or antibody fragment is bound to a solid support.

10. The method of claim 1 wherein the label of the labeled antibody or antibody fragment is selected from the group consisting of a chemiluminescent agent, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

11. The method of claim 1, wherein the first antibody or antibody fragment is a goat anti-(1-6) parathyroid hormone antibody.

12. The method of claim 1, wherein the method is capable of detecting wPTH at a normal physiological level.

13. The method of claim 1, wherein the method is capable of detecting wPTH at levels of 27.89 pg/ml and below.

14. The method of claim 1, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

15. The method of claim 1, further comprising the step of determining either the level of total PTH or the level of parathyroid hormone inhibitory peptide fragment or the level of both in the sample.

16. The method of claim 15, wherein the level of parathyroid hormone inhibitory peptide fragment in the sample is determined by subtracting the measured level of whole PTH in the sample from the measured level of total PTH in the sample to calculate the level of parathyroid hormone inhibitory peptide fragment.

17. The method of claim 15 or 16, wherein total PTH level is determined using an antibody specific for the fragment PTH_{7-38}.

18. The method of claim 15 or 16, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby determining whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

19. The method of claim 18, wherein the parathyroid disease is primary hyperparathyroidism.

20. The method of claim 18, wherein the parathyroid disease is secondary hyperparathyroidism.

21. The method of claim 18, wherein the parathyroid disease is caused by chronic renal failure.

22. The method of claim 18, wherein the parathyroid disease is renal osteodystrophy.

23. The method of claim 22, wherein the renal osteodystrophy is selected from the group consisting of osteitis fibrosa

cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

24. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the parathyroid hormone inhibitory peptide fragment level.

25. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the total parathyroid hormone level in the sample.

26. The method of Claim 18 wherein the parathyroid hormone inhibitory peptide fragment level is compared with the total parathyroid hormone level in the sample.

27. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level,
thereby monitoring parathyroid related bone disease and treatment in the person from whom the sample was collected.

28. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby monitoring effects of the therapeutic treatment for hyperparathyroidism in the person from whom the sample was collected.

29. The method of claim 28, wherein the hyperparathyroidism is selected from the group consisting of primary hyperparathyroidism, secondary hyperparathyroidism, renal bone disease, renal osteodystrophy, osteitis fibrosa cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

30. The method of any of claims 15-17, further comprising the step of comparing the whole parathyroid hormone level with the parathyroid hormone inhibitory peptide fragment level to monitor renal osteodystrophy and its treatment.

31. The method of any of claims 18-30 wherein the comparison is in the form of a ratio or proportion.

32. The method of claim 18, wherein the sample is from a person who is a patient with chronic uremia.

33. The method of claim 31, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

34. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence from between PTH_{3-84} (SEQ ID NO:2) and PTH_{34-84} (SEQ ID NO:3) and functions *in vivo* as a parathyroid hormone antagonist or inhibitor (PIN).

35. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

36. The method of claim 1, further comprising the step of using the level of whole parathyroid hormone in the sample to determine whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

37. A substantially pure antibody or antibody fragment specific for an initial peptide sequence of whole parathyroid hormone, wherein the initial peptide sequence consists of SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO: 4) as part of wPTH, wherein at least four amino acids in this sequence are part of a reactive portion with the antibody or antibody fragment, and wherein the antibody or antibody fragment is produced using the complete wPTH peptidic sequence as an immunogen, and wherein the antibody or antibody fragment does not detect an intact (or) non-(1-84) parathyroid hormone fragment

38. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a monoclonal antibody.

39. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a polyclonal antibody.

40. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of

detecting wPTH at a normal physiological level.

41. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of detecting wPTH at levels of 27.89 pg/ml and below.

42. The antibody or antibody fragment of any of claims 37-41, or the method of any of claims 1 to 36, wherein the first antibody or antibody fragment is affinity purified using a synthetic peptide selected from hPTH1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET), rat PTH 1-8 (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)), or a peptide of at least four amino acids in the common sequence.

Patentansprüche

1. Verfahren zum Messen der Menge an vollständigem Parathormon in einer Probe, während ein interferierendes Nicht-(1-84)-Parathormonfragment nicht detektiert wird, wobei das Verfahren **gekennzeichnet ist durch:**

   a) Zugabe zur Probe eines ersten Antikörpers oder Antikörperfragments, welches für das Parathormonpeptid SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil der Komplettsequenz des vollständigen Parathormons (wPTH) spezifisch ist, und wobei zumindest vier Aminosäuren in dem Peptid Teil eines reaktiven Bereichs des ersten Antikörpers oder Antikörperfragments sind, wobei der erste Antikörper oder das erste Antikörperfragment unter Verwendung der kompletten wPTH-Peptidsequenz als ein Immunogen hergestellt wird,
   b) Zugabe eines zweiten Antikörpers oder Antikörperfragments, der/das spezifisch an einem anderen Teil des vollständigen Parathormons als die anfängliche Parathormon-Peptidsequenz, die an den ersten Antikörper bindet, bindet,
   wobei entweder erster/s Antikörper oder Antikörperfragment oder der zweite/s Antikörper oder Antikörperfragment markiert ist,
   wodurch ein markierter Komplex gebildet wird; und
   c) Messen der Menge des markierten Komplexes, um die Menge an vollständigem Parathormon in der Probe zu messen.

2. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment sequentiell oder gleichzeitig mit dem ersten Antikörper oder Antikörperfragment zugegeben wird.

3. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment an einem festen Träger gebunden ist.

4. Verfahren nach Anspruch 3, wobei der/das erste Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

5. Verfahren nach Anspruch 4, wobei der kolloidale, feste Träger Latexpartikel ist.

6. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein monoklonaler Antikörper ist.

7. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein polyklonaler Antikörper ist.

8. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment markiert ist.

9. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

10. Verfahren nach Anspruch 1, wobei die Markierung des markierten Antikörpers oder Antikörperfragments ausgewählt ist aus der Gruppe, die aus einem Chemilumineszenzagens, einen kolorimetrischen Agens, einem Energieübertragungsagens, einem Enzym, einem Fluoreszenzagens und einem Radioisotop besteht.

11. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment ein Ziegen-anti-(1-6)-Para-

THIRD AUXILIARY REQUEST


```
Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln
1               5                   10                  15

Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys
            20                  25                  30

Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala
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Lys Ser Gln
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Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg Val Glu
1               5                   10                  15

Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
            20                  25                  30
```

Claims

1. A method for measuring the amount of whole parathyroid hormone in a sample while not detecting an interfering non-(1-84) parathyroid hormone fragment, said method **characterized by**:

   a) adding to the sample a first antibody or antibody fragment specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment; which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen;

   b) adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the initial parathyroid hormone peptide sequence which binds to the first antibody, wherein either the first antibody or antibody fragment or the second antibody or antibody fragment is labeled, thereby forming a labeled complex; and

c) measuring the amount of the labeled complex to measure the amount of whole parathyroid hormone in the sample.

2. The method of claim 1 wherein the second antibody or antibody fragment is added sequentially or simultaneously with the first antibody or antibody fragment.

3. The method of claim 1 wherein the first antibody or antibody fragment is bound to a solid support.

4. The method of claim 3 wherein the first antibody or antibody fragment is bound to a colloidal solid support.

5. The method of claim 4 wherein the colloidal solid support is latex particles.

6. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a monoclonal antibody.

7. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a polyclonal antibody.

8. The method of claim 1, wherein the second antibody or antibody fragment is labeled.

9. The method of claim 1 wherein the second antibody or antibody fragment is bound to a solid support.

10. The method of claim 1 wherein the label of the labeled antibody or antibody fragment is selected from the group consisting of a chemiluminescent agent, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

11. The method of claim 1, wherein the first antibody or antibody fragment is a goat anti-(1-6) parathyroid hormone antibody.

12. The method of claim 1, wherein the method is capable of detecting wPTH at a normal physiological level.

~~13. The method of claim 1, wherein the method is capable of detecting wPTH at levels of 27.89 pg/ml and below.~~

13 ~~14.~~ The method of claim 1, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

14 ~~15.~~ The method of claim 1, further comprising the step of determining either the level of total PTH or the level of parathyroid hormone inhibitory peptide fragment or the level of both in the sample.

15 ~~16.~~ The method of claim ~~15~~ 14, wherein the level of parathyroid hormone inhibitory peptide fragment in the sample is determined by subtracting the measured level of whole PTH in the sample from the measured level of total PTH in the sample to calculate the level of parathyroid hormone inhibitory peptide fragment.

16 ~~17.~~ The method of claim ~~16~~ 14 or ~~16~~ 15, wherein total PTH level is determined using an antibody specific for the fragment PTH_{7-38}.

17 ~~18.~~ The method of claim ~~15~~ 14 or ~~16~~ 15, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby determining whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

18 ~~19.~~ The method of claim ~~18~~ 17, wherein the parathyroid disease is primary hyperparathyroidism.

19 ~~20.~~ The method of claim ~~18~~ 17, wherein the parathyroid disease is secondary hyperparathyroidism.

20 ~~21.~~ The method of claim ~~18~~ 17, wherein the parathyroid disease is caused by chronic renal failure.

21 ~~22.~~ The method of claim ~~18~~ 17, wherein the parathyroid disease is renal osteodystrophy.

22 ~~23.~~ The method of claim ~~22~~ 21, wherein the renal osteodystrophy is selected from the group consisting of osteitis fibrosa

cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

24. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the parathyroid hormone inhibitory peptide fragment level.

25. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the total parathyroid hormone level in the sample.

26. The method of Claim 18 wherein the parathyroid hormone inhibitory peptide fragment level is compared with the total parathyroid hormone level in the sample.

27. The method of any of claims 18-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level,
thereby monitoring parathyroid related bone disease and treatment in the person from whom the sample was collected.

28. The method of any of claims 18-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby monitoring effects of the therapeutic treatment for hyperparathyroidism in the person from whom the sample was collected.

29. The method of claim 28, wherein the hyperparathyroidism is selected from the group consisting of primary hyperparathyroidism, secondary hyperparathyroidism, renal bone disease, renal osteodystrophy, osteitis fibrosa cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

30. The method of any of claims 15-17, further comprising the step of comparing the whole parathyroid hormone level with the parathyroid hormone inhibitory peptide fragment level to monitor renal osteodystrophy and its treatment.

31. The method of any of claims 18-30 wherein the comparison is in the form of a ratio or proportion.

32. The method of claim 18, wherein the sample is from a person who is a patient with chronic uremia.

33. The method of claim 31, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

34. The method of claim 18, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence from between PTH_{3-84} (SEQ ID NO:2) and PTH_{34-84} (SEQ ID NO:3) and functions *in vivo* as a parathyroid hormone antagonist or inhibitor (PIN).

35. The method of claim 18, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

36. The method of claim 1, further comprising the step of using the level of whole parathyroid hormone in the sample to determine whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

37. A substantially pure antibody or antibody fragment specific for an initial peptide sequence of whole parathyroid hormone, wherein the initial peptide sequence consists of SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO: 4) as part of wPTH, wherein at least four amino acids in this sequence are part of a reactive portion with the antibody or antibody fragment, and wherein the antibody or antibody fragment is produced using the complete wPTH peptidic sequence as an immunogen.

38. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a monoclonal antibody.

39. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a polyclonal antibody.

40. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of

detecting wPTH at a normal physiological level.

~~41. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of detecting wPTH at levels of 27.89 pg/ml and below.~~

42. The antibody or antibody fragment of any of claims 37-41, or the method of any of claims 1 to 36, wherein the first antibody or antibody fragment is affinity purified using a synthetic peptide selected from hPTH1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET), rat PTH 1-8 (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)), or a peptide of at least four amino acids in the common sequence.

Patentansprüche

1. Verfahren zum Messen der Menge an vollständigem Parathormon in einer Probe, während ein interferierendes Nicht-(1-84)-Parathormonfragment nicht detektiert wird, wobei das Verfahren **gekennzeichnet ist durch**:

   a) Zugabe zur Probe eines ersten Antikörpers oder Antikörperfragments, welches für das Parathormonpeptid SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil der Komplettsequenz des vollständigen Parathormons (wPTH) spezifisch ist, und wobei zumindest vier Aminosäuren in dem Peptid Teil eines reaktiven Bereichs des ersten Antikörpers oder Antikörperfragments sind, wobei der erste Antikörper oder das erste Antikörperfragment unter Verwendung der kompletten wPTH-Peptidsequenz als ein Immunogen hergestellt wird,
   b) Zugabe eines zweiten Antikörpers oder Antikörperfragments, der/das spezifisch an einem anderen Teil des vollständigen Parathormons als die anfängliche Parathormon-Peptidsequenz, die an den ersten Antikörper bindet, bindet,
   wobei entweder erster/s Antikörper oder Antikörperfragment oder der zweite/s Antikörper oder Antikörperfragment markiert ist,
   wodurch ein markierter Komplex gebildet wird; und
   c) Messen der Menge des markierten Komplexes, um die Menge an vollständigem Parathormon in der Probe zu messen.

2. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment sequentiell oder gleichzeitig mit dem ersten Antikörper oder Antikörperfragment zugegeben wird.

3. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment an einem festen Träger gebunden ist.

4. Verfahren nach Anspruch 3, wobei der/das erste Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

5. Verfahren nach Anspruch 4, wobei der kolloidale, feste Träger Latexpartikel ist.

6. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein monoklonaler Antikörper ist.

7. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein polyklonaler Antikörper ist.

8. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment markiert ist.

9. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

10. Verfahren nach Anspruch 1, wobei die Markierung des markierten Antikörpers oder Antikörperfragments ausgewählt ist aus der Gruppe, die aus einem Chemilumineszenzagens, einen kolorimetrischen Agens, einem Energieübertragungsagens, einem Enzym, einem Fluoreszenzagens und einem Radioisotop besteht.

11. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment ein Ziegen-anti-(1-6)-Para-

Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln
1 5 10 15

Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys
20 25 30

Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala
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Lys Ser Gln
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Ser Val Ser Glu Ile Gln Leu Met
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<213> Homo sapiens

<400> 5

Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg Val Glu
1 5 10 15

Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
20 25 30

Claims

1. A method for measuring the amount of whole parathyroid hormone in a sample while not detecting an interfering non-(1-84) parathyroid hormone fragment, said method **characterized by**:

a) adding to the sample a first antibody or antibody fragment specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment; which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen;

b) adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the Initial parathyroid hormone peptide sequence which binds to the first antibody, wherein either the first antibody or antibody fragment or the second antibody or antibody fragment is labeled, thereby forming a labeled complex; and

c) measuring the amount of the labeled complex to measure the amount of whole parathyroid hormone in the sample.

INSERT < > FROM CLAIM 12

2. The method of claim 1 wherein the second antibody or antibody fragment is added sequentially or simultaneously with the first antibody or antibody fragment.

3. The method of claim 1 wherein the first antibody or antibody fragment is bound to a solid support.

4. The method of claim 3 wherein the first antibody or antibody fragment is bound to a colloidal solid support.

5. The method of claim 4 wherein the colloidal solid support is latex particles.

6. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a monoclonal antibody.

7. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a polyclonal antibody.

8. The method of claim 1, wherein the second antibody or antibody fragment is labeled.

9. The method of claim 1 wherein the second antibody or antibody fragment is bound to a solid support.

10. The method of claim 1 wherein the label of the labeled antibody or antibody fragment is selected from the group consisting of a chemiluminescent agent, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

11. The method of claim 1, wherein the first antibody or antibody fragment is a goat anti-(1-6) parathyroid hormone antibody.

~~12. The method of claim 1~~ < wherein the method is capable of detecting wPTH at a normal physiological level. >

~~13. The method of claim 1, wherein the method is capable of detecting wPTH at levels of 27.89 pg/ml and below.~~

12 ~~14~~. The method of claim 1, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

13 ~~15~~. The method of claim 1, further comprising the step of determining either the level of total PTH or the level of parathyroid hormone inhibitory peptide fragment or the level of both in the sample.

14 ~~16~~. The method of claim 13 ~~15~~, wherein the level of parathyroid hormone inhibitory peptide fragment in the sample is determined by subtracting the measured level of whole PTH in the sample from the measured level of total PTH in the sample to calculate the level of parathyroid hormone inhibitory peptide fragment.

15 ~~17~~. The method of claim 13 ~~15~~ or 14 ~~16~~, wherein total PTH level is determined using an antibody specific for the fragment PTH_{7-38}.

16 ~~18~~. The method of claim 13 ~~15~~ or 14 ~~16~~, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby determining whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

17 ~~19~~. The method of claim 16 ~~18~~, wherein the parathyroid disease is primary hyperparathyroidism.

18 ~~20~~. The method of claim 16 ~~18~~, wherein the parathyroid disease is secondary hyperparathyroidism.

19 ~~21~~. The method of claim 16 ~~18~~, wherein the parathyroid disease is caused by chronic renal failure.

20 ~~22~~. The method of claim 16 ~~18~~, wherein the parathyroid disease is renal osteodystrophy.

21 ~~23~~. The method of claim 20 ~~22~~, wherein the renal osteodystrophy is selected from the group consisting of osteitis fibrosa

cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

22 ~~24~~. The method of Claim ~~18~~ 16 wherein the whole parathyroid hormone level is compared with the parathyroid hormone inhibitory peptide fragment level.

23 ~~25~~. The method of Claim ~~18~~ 16 wherein the whole parathyroid hormone level is compared with the total parathyroid hormone level in the sample.

24 ~~26~~. The method of Claim ~~18~~ 16 wherein the parathyroid hormone inhibitory peptide fragment level is compared with the total parathyroid hormone level in the sample.

25 ~~27~~. The method of any of claims ~~15-17~~ 13 15, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level,
thereby monitoring parathyroid related bone disease and treatment in the person from whom the sample was collected.

26 ~~28~~. The method of any of claims ~~15-17~~ 13 15, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby monitoring effects of the therapeutic treatment for hyperparathyroidism in the person from whom the sample was collected.

27 ~~29~~. The method of claim ~~28~~ 26, wherein the hyperparathyroidism is selected from the group consisting of primary hyperparathyroidism, secondary hyperparathyroidism, renal bone disease, renal osteodystrophy, osteitis fibrosa cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

28 ~~30~~. The method of any of claims ~~15-17~~ 13 15, further comprising the step of comparing the whole parathyroid hormone level with the parathyroid hormone inhibitory peptide fragment level to monitor renal osteodystrophy and its treatment.

29 ~~31~~. The method of any of claims ~~18-30~~ 16 28 wherein the comparison is in the form of a ratio or proportion.

30 ~~32~~. The method of claim ~~18~~ 16, wherein the sample is from a person who is a patient with chronic uremia.

31 ~~33~~. The method of claim ~~31~~ 29, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

32 ~~34~~. The method of claim ~~18~~ 13, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence from between PTH_{3-84} (SEQ ID NO:2) and PTH_{34-84} (SEQ ID NO:3) and functions *in vivo* as a parathyroid hormone antagonist or inhibitor (PIN).

33 ~~35~~. The method of claim ~~18~~ 13, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

34 ~~36~~. The method of claim 1, further comprising the step of using the level of whole parathyroid hormone in the sample to determine whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

35 ~~37~~. A substantially pure antibody or antibody fragment specific for an initial peptide sequence of whole parathyroid hormone, wherein the initial peptide sequence consists of SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO: 4) as part of wPTH, wherein at least four amino acids in this sequence are part of a reactive portion with the antibody or antibody fragment, and wherein the antibody or antibody fragment is produced using the complete wPTH peptidic sequence as an immunogen and is capable of detecting wPTH in a sample at normal physiological levels and does not detect an interfering non-(1-84) parathyroid hormone fragment.

36 ~~38~~. The antibody or antibody fragment of claim ~~37~~ 35 wherein the antibody or antibody fragment is a monoclonal antibody.

37 ~~39~~. The antibody or antibody fragment of claim ~~37~~ 35 wherein the antibody or antibody fragment is a polyclonal antibody.

~~40. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of~~

~~detecting wPTH at a normal physiological level.~~

41. ~~The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of detecting wPTH at levels of 27.89 pg/ml and below.~~

38 42. The antibody or antibody fragment of any of claims 35-37, or the method of any of claims 1 to 36, wherein the first antibody or antibody fragment is affinity purified using a synthetic peptide selected from hPTH1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET), rat PTH 1-8 (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)), or a peptide of at least four amino acids in the common sequence.

Patentansprüche

1. Verfahren zum Messen der Menge an vollständigem Parathormon in einer Probe, während ein interferierendes Nicht-(1-84)-Parathormonfragment nicht detektiert wird, wobei das Verfahren **gekennzeichnet ist durch:**

   a) Zugabe zur Probe eines ersten Antikörpers oder Antikörperfragments, welches für das Parathormonpeptid SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil der Komplettsequenz des vollständigen Parathormons (wPTH) spezifisch ist, und wobei zumindest vier Aminosäuren in dem Peptid Teil eines reaktiven Bereichs des ersten Antikörpers oder Antikörperfragments sind, wobei der erste Antikörper oder das erste Antikörperfragment unter Verwendung der kompletten wPTH-Peptidsequenz als ein Immunogen hergestellt wird,
   b) Zugabe eines zweiten Antikörpers oder Antikörperfragments, der/das spezifisch an einem anderen Teil des vollständigen Parathormons als die anfängliche Parathormon-Peptidsequenz, die an den ersten Antikörper bindet, bindet,
   wobei entweder erster/s Antikörper oder Antikörperfragment oder der zweite/s Antikörper oder Antikörperfragment markiert ist,
   wodurch ein markierter Komplex gebildet wird; und
   c) Messen der Menge des markierten Komplexes, um die Menge an vollständigem Parathormon in der Probe zu messen.

2. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment sequentiell oder gleichzeitig mit dem ersten Antikörper oder Antikörperfragment zugegeben wird.

3. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment an einem festen Träger gebunden ist.

4. Verfahren nach Anspruch 3, wobei der/das erste Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

5. Verfahren nach Anspruch 4, wobei der kolloidale, feste Träger Latexpartikel ist.

6. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein monoklonaler Antikörper ist.

7. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein polyklonaler Antikörper ist.

8. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment markiert ist.

9. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

10. Verfahren nach Anspruch 1, wobei die Markierung des markierten Antikörpers oder Antikörperfragments ausgewählt ist aus der Gruppe, die aus einem Chemilumineszenzagens, einen kolorimetrischen Agens, einem Energieübertragungsagens, einem Enzym, einem Fluoreszenzagens und einem Radioisotop besteht.

11. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment ein Ziegen-anti-(1-6)-Para-

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Phe Val Ala Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser Gln
1               5                   10                  15

Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val Glu Ser His Glu Lys
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Ser Leu Gly Glu Ala Asp Lys Ala Asp Val Asn Val Leu Thr Lys Ala
        35                  40                  45

Lys Ser Gln
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<400> 5

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Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg Val Glu
1               5                   10                  15

Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu Gly
            20                  25                  30
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Claims

1. A method for measuring the amount of whole parathyroid hormone in a sample while not detecting an interfering non-(1-84) parathyroid hormone fragment, said method **characterized by**:

   a) adding to the sample a first antibody or antibody fragment specific for the parathyroid hormone peptide SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) as part of whole parathyroid hormone (wPTH) complete sequence, and wherein at least four amino acids in said peptide are part of a reactive portion to said first antibody or antibody fragment; which first antibody or antibody fragment was produced using the complete wPTH peptidic sequence as an immunogen;

   b) adding a second antibody or antibody fragment that specifically binds to a portion of whole parathyroid hormone other than the initial parathyroid hormone peptide sequence which binds to the first antibody, wherein either the first antibody or antibody fragment or the second antibody or antibody fragment is labeled, thereby forming a labeled complex; and

c) measuring the amount of the labeled complex to measure the amount of whole parathyroid hormone in the sample.

2. The method of claim 1 wherein the second antibody or antibody fragment is added sequentially or simultaneously with the first antibody or antibody fragment.

3. The method of claim 1 wherein the first antibody or antibody fragment is bound to a solid support.

4. The method of claim 3 wherein the first antibody or antibody fragment is bound to a colloidal solid support.

5. The method of claim 4 wherein the colloidal solid support is latex particles.

6. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a monoclonal antibody.

7. The method of claim 1 wherein the first antibody or antibody fragment is labeled and is a polyclonal antibody.

8. The method of claim 1, wherein the second antibody or antibody fragment is labeled.

9. The method of claim 1 wherein the second antibody or antibody fragment is bound to a solid support.

10. The method of claim 1 wherein the label of the labeled antibody or antibody fragment is selected from the group consisting of a chemiluminescent agent, a colorimetric agent, an energy transfer agent, an enzyme, a fluorescent agent, and a radioisotope.

11. The method of claim 1, wherein the first antibody or antibody fragment is a goat anti-(1-6) parathyroid hormone antibody.

12. The method of claim 1, wherein the method is capable of detecting wPTH at a normal physiological level.

13. The method of claim 1, wherein the method is capable of detecting wPTH at levels of 27.89 pg/ml and below.

14. The method of claim 1, wherein the sample is selected from the group consisting of a serum, a plasma and a blood sample.

15. The method of claim 1, further comprising the step of determining either the level of total PTH or the level of parathyroid hormone inhibitory peptide fragment or the level of both in the sample.

16. The method of claim 15, wherein the level of parathyroid hormone inhibitory peptide fragment in the sample is determined by subtracting the measured level of whole PTH in the sample from the measured level of total PTH in the sample to calculate the level of parathyroid hormone inhibitory peptide fragment.

17. The method of claim 15 or 16, wherein total PTH level is determined using an antibody specific for the fragment PTH_{7-38}.

18. The method of claim 15 or 16, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby determining whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

19. The method of claim 18, wherein the parathyroid disease is primary hyperparathyroidism.

20. The method of claim 18, wherein the parathyroid disease is secondary hyperparathyroidism.

21. The method of claim 18, wherein the parathyroid disease is caused by chronic renal failure.

22. The method of claim 18, wherein the parathyroid disease is renal osteodystrophy.

23. The method of claim 22, wherein the renal osteodystrophy is selected from the group consisting of osteitis fibrosa

cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

24. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the parathyroid hormone inhibitory peptide fragment level.

25. The method of Claim 18 wherein the whole parathyroid hormone level is compared with the total parathyroid hormone level in the sample.

26. The method of Claim 18 wherein the parathyroid hormone inhibitory peptide fragment level is compared with the total parathyroid hormone level in the sample.

27. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level,
thereby monitoring parathyroid related bone disease and treatment in the person from whom the sample was collected.

28. The method of any of claims 15-17, further comprising the step of comparing at least two parameters selected from the group consisting of the whole parathyroid hormone level, parathyroid hormone inhibitory peptide fragment level, and total parathyroid hormone level, thereby monitoring effects of the therapeutic treatment for hyperparathyroidism in the person from whom the sample was collected.

29. The method of claim 28, wherein the hyperparathyroidism is selected from the group consisting of primary hyperparathyroidism, secondary hyperparathyroidism, renal bone disease, renal osteodystrophy, osteitis fibrosa cystica, osteomalacia, extraskeletal calcification/ossification and an adynamic bone disease.

30. The method of any of claims 15-17, further comprising the step of comparing the whole parathyroid hormone level with the parathyroid hormone inhibitory peptide fragment level to monitor renal osteodystrophy and its treatment.

31. The method of any of claims 18-30 wherein the comparison is in the form of a ratio or proportion.

32. The method of claim 18, wherein the sample is from a person who is a patient with chronic uremia.

33. The method of claim 31, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

34. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence from between PTH_{3-84} (SEQ ID NO:2) and PTH_{34-84} (SEQ ID NO:3) and functions *in vivo* as a parathyroid hormone antagonist or inhibitor (PIN).

35. The method of claim 15, wherein the parathyroid hormone inhibitory peptide fragment is a peptide having an amino acid sequence of human PTH_{7-84}.

36. The method of claim 1, further comprising the step of using the level of whole parathyroid hormone in the sample to determine whether the sample is from a person who has substantially normal parathyroid function or has a parathyroid disease.

37. A substantially pure antibody or antibody fragment specific for an initial peptide sequence of whole parathyroid hormone, wherein the initial peptide sequence consists of SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO: 4) as part of wPTH, wherein at least four amino acids in this sequence are part of a reactive portion with the antibody or antibody fragment, and wherein the antibody or antibody fragment is produced using the complete wPTH peptidic sequence as an immunogen.

38. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a monoclonal antibody.

39. The antibody or antibody fragment of claim 37 wherein the antibody or antibody fragment is a polyclonal antibody.

40. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of

detecting wPTH at a normal physiological level.

41. The antibody or antibody fragment of any of claims 37-39, wherein the antibody or antibody fragment is capable of detecting wPTH at levels of 27.89 pg/ml and below.

42. The antibody or antibody fragment of any of claims 37-41, or the method of any of claims 1 to 36, wherein the first antibody or antibody fragment is affinity purified using a synthetic peptide selected from hPTH1-8 (SER-VAL-SER-GLU-ILE-GLN-LEU-MET) or rat PTH 1-8 (ALA-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4)), ~~or a peptide of at least four amino acids in the common sequence.~~

43. < > peptide with at least four amino acids in the common sequence of hPTH 1-8 and rat PTH 1-8.

Patentansprüche

1. Verfahren zum Messen der Menge an vollständigem Parathormon in einer Probe, während ein interferierendes Nicht-(1-84)-Parathormonfragment nicht detektiert wird, wobei das Verfahren **gekennzeichnet ist durch:**

   a) Zugabe zur Probe eines ersten Antikörpers oder Antikörperfragments, welches für das Parathormonpeptid SER-VAL-SER-GLU-ILE-GLN-LEU-MET (SEQ ID NO:4) als Teil der Komplettsequenz des vollständigen Parathormons (wPTH) spezifisch ist, und wobei zumindest vier Aminosäuren in dem Peptid Teil eines reaktiven Bereichs des ersten Antikörpers oder Antikörperfragments sind, wobei der erste Antikörper oder das erste Antikörperfragment unter Verwendung der kompletten wPTH-Peptidsequenz als ein Immunogen hergestellt wird,
   b) Zugabe eines zweiten Antikörpers oder Antikörperfragments, der/das spezifisch an einem anderen Teil des vollständigen Parathormons als die anfängliche Parathormon-Peptidsequenz, die an den ersten Antikörper bindet, bindet,
   wobei entweder erster/s Antikörper oder Antikörperfragment oder der zweite/s Antikörper oder Antikörperfragment markiert ist,
   wodurch ein markierter Komplex gebildet wird; und
   c) Messen der Menge des markierten Komplexes, um die Menge an vollständigem Parathormon in der Probe zu messen.

2. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment sequentiell oder gleichzeitig mit dem ersten Antikörper oder Antikörperfragment zugegeben wird.

3. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment an einem festen Träger gebunden ist.

4. Verfahren nach Anspruch 3, wobei der/das erste Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

5. Verfahren nach Anspruch 4, wobei der kolloidale, feste Träger Latexpartikel ist.

6. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein monoklonaler Antikörper ist.

7. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment markiert ist und ein polyklonaler Antikörper ist.

8. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment markiert ist.

9. Verfahren nach Anspruch 1, wobei der/das zweite Antikörper oder Antikörperfragment an einem kolloidalen, festen Träger gebunden ist.

10. Verfahren nach Anspruch 1, wobei die Markierung des markierten Antikörpers oder Antikörperfragments ausgewählt ist aus der Gruppe, die aus einem Chemilumineszenzagens, einen kolorimetrischen Agens, einem Energieübertragungsagens, einem Enzym, einem Fluoreszenzagens und einem Radioisotop besteht.

11. Verfahren nach Anspruch 1, wobei der/das erste Antikörper oder Antikörperfragment ein Ziegen-anti-(1-6)-Para-